Phenotypic screening identifies HDAC6 inhibitors as cardioprotective agents in BAG3 cardiac-knockout mouse model of dilated cardiomyopathy

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Use of iPSC-CM as Drug Discovery Platform For Dilated Cardiomyopathy

**Background:** Familial dilated cardiomyopathy (DCM) is characterized by reduced cardiac output, as well as thinning and enlargement of left ventricular chambers. These characteristics eventually lead to heart failure. Current standards of care do not target the underlying molecular mechanisms associated with genetic forms of heart failure, driving a need to develop novel therapeutics that target genetic DCM.

**Purpose:** To identify candidate therapeutics, we developed an in vitro DCM model using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) deficient in BAG3.

**Methods and Results:** Using BAG3-deficient iPSC-CMs, we identified cardioprotective drugs with a phenotypic screen and deep learning. Using a library of 5500 bioactive compounds and siRNA validation, we identified that inhibiting HDAC6 was cardioprotective at the sarcomere level. We translated this finding to a BAG3 cardiac-knockout (BAG3<sup>−/−</sup>) mouse model of DCM, showing that inhibiting HDAC6 with two isoform-selective inhibitors (tubastatin A and a novel inhibitor TYA-018) protected heart function. HDAC6 inhibitors also protected the microtubule network from mechanical damage, increased autophagic flux, decreased apoptosis, and reduced inflammation in the heart.

**Conclusion:** HDAC6 inhibitors improved left ventricular ejection fraction and extended lifespan in a BAG3<sup>−/−</sup> mouse model of DCM. Our results demonstrate the power of combining iPSC-CMs with phenotypic screening and deep learning to identify therapeutic targets for DCM.

Target Validation Shows Inhibiting HDAC6 is Sufficient to Protect Against Sarcomere Damage

(A) Schematic of drug treatment in BAG3<sup>−/−</sup> mouse model. TYA-018 (highly selective HDAC6 inhibitor) was administered daily by oral gavage at 15 mg/kg starting when mice were 2 months of age.

(B) Daily dosing of TYA-018 protected heart function during the 8-week dosing period as measured by ejection fraction. Error bars = SEM. **P < 0.01.

(C) Ejection fraction was tracked from the first day of dosing, and delta ejection fraction was measured. During the 8-week period, heart function did not decline in the TYA-018-treated arm, whereas it dropped by 19.1% in the vehicle-treated arm. Error bars = SEM. **P < 0.01.

(D & E) Ejection fraction (D) and delta ejection fraction (E) (compared to the pre-dose baseline) at 4 months of age and 8 weeks of dosing shows TYA-018 protects against declining heart function in BAG3<sup>−/−</sup> mice. Error bars = SEM. **P < 0.01.

(Veh, vehicle; F & G) Left ventricular internal diameter at diastole (LVdD) (F) and systole (LVdS) (G) were reduced by TYA-018 in BAG3<sup>−/−</sup> mice, bringing the levels closer to that of their wild-type (WT) littermates. Error bars = SEM. *P < 0.05.

High throughput Screening Identifies HDAC and Microtubule Inhibitors a Cardioprotective Agents

(A) An unbiased screen was performed using a library of 5500 bioactive compounds. iPSC-CMs were treated with BAG3 siRNA and compounds at a concentration of 1µM. Hits were first identified using deep learning on control iPSC-CMs treated with either SCR or BAG3 siRNA. The hit threshold was set at a cardiomyocyte score of 0.3. (C) The top 24 compounds consisted of histone deacetylase (HDAC) and microtubule inhibitors. In addition, three known heart failure agents were identified: sotalol (beta-blocker and K-channel blocker), omecamtiv mecarbil (cardiac myosin activator), and anagrelide (PDE3 inhibitor).

Inhibiting HDAC6 with TYA-018 Protects Heart Function in BAG3<sup>−/−</sup> Mice

(A) RNA-Seq analysis shows NPPB expression increased by approximately fourfold in BAG3<sup>−/−</sup> mice compared to WT mice at 4 months of age, TYA-018 treatment reduced NPPB levels by twofold in BAG3<sup>−/−</sup> mice. The level of NPPB in BAG3<sup>−/−</sup> mice was anticorrelated with heart function.

(C) The top terms in the biological process category enriched in BAG3<sup>−/−</sup> mice treated with TYA-018. These genes were selected based on positive correlation (R>0.5) with cardiac function (EF%) and negative correlation (R<-0.5) with NPPB expression.

(D) Heatmap shows genes from the selected GO terms.